

Claims

What is claimed is:

5        1. A method for the automated identification of a nucleic acid ligand from a candidate mixture of nucleic acids, said nucleic acid ligand being a ligand of a given target comprising:

a) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

10      b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids, wherein a nucleic acid ligand is identified, wherein steps (a)-(c) are performed at one or more work stations on a work surface by a cartesian robotic manipulator controlled by a computer.

15      2. The method of claim 1 further comprising the step:

d) repeating steps a) through c) using the ligand enriched mixture of each successive repeat as many times as required to yield a desired level of increased ligand enrichment.

20      3. The method of claim 2 wherein said target is attached to a solid support, and wherein step (b) is accomplished by partitioning said solid support from said candidate mixture.

25      4. The method of claim 3 wherein said solid support is a multi-well microtitre plate.

30      5. The method of claim 4 wherein said plate is comprised of polystyrene.

6. The method of claim 5 wherein said target is attached to said plate by hydrophobic interactions.

7. The method of claim 3 wherein said solid support is a paramagnetic bead and wherein the partitioning of said paramagnetic bead is performed by a magnetic bead separator controlled by said computer.

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8. The method of claim 1 wherein said candidate nucleic acid ligands have fixed sequence regions, and wherein step (c) is performed using the polymerase chain reaction with primers complementary to said fixed sequence regions.

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9. The method of claim 8 wherein said computer makes a measurement of the amount of amplified product and calculates a value for the initial concentration of nucleic acid ligand eluted from target using said measurement.

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10. The method of claim 9 wherein said computer adjusts the reaction conditions of steps (a)-(c) in a predetermined manner in response to said value.

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11. The method of claim 9 wherein said primers are labeled with fluorophores and quenching groups at nucleotide positions that move relative to one another when said primers become incorporated into amplified product, such that the fluorescence emission profiles of said primers change upon incorporation into amplified product, and wherein said computer makes the measurement of the amount of amplified product by detecting said change.

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12. The method of claim 11 said computer controls a fluorescence detection means, and wherein said computer operates said detection means to make the measurement of the amount of amplified product.

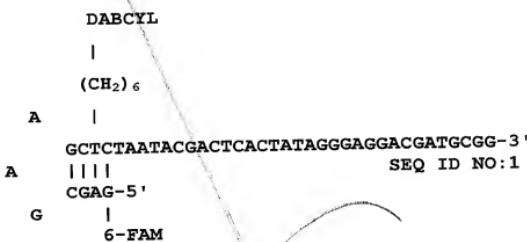
13. The method of claim 11 wherein at least one of said primers comprises:

(a) a single stranded DNA molecule complementary to one of said fixed sequence regions;

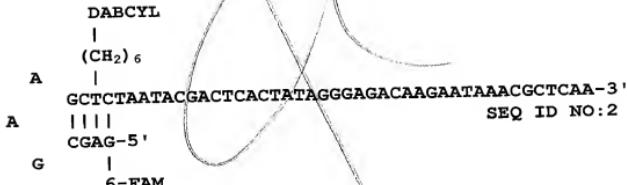
(b) a stem-loop structure attached to the 5' end of said single stranded DNA molecule, said stem comprising a fluorophore and a quenching agent located at nucleotide positions on opposite sides of the stem of said stem-loop structure, said nucleotide positions located sufficiently close to one another such that the fluorescent signal from said fluorophore is substantially quenched by said quenching agent;

5 wherein the extension of the 3' end of candidate nucleic acid ligands that anneal to said primer during said polymerase chain reaction disrupts said stem structure, wherein said fluorescent group is no longer quenched by said quenching group.

10 14. The method of claim 13 wherein said primer is selected from the group consisting of:



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35 18. An improved method for the identification of a nucleic acid ligand from a candidate mixture of nucleic acids, said nucleic acid ligand being a ligand of a given target, wherein said method comprises:

- a) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;
- b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
- c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids,
- the improvement comprising:
- performing steps (a)-(c) substantially by automated machines controlled by a computer.

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16. A method for the automated identification of a nucleic acid ligand from a candidate mixture of nucleic acids, said nucleic acid ligand being a ligand of a given target comprising:

- a) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;
- b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and
- c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids,
- wherein steps (a)-(c) are carried out substantially by automated machines controlled by a computer.

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17. An automated machine carrying out the method of claim 1.

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- Sub a?* X 18. A method for identifying a nucleic acid ligand that photocrosslinks to a protein from a candidate mixture of nucleic acids, wherein each member of said candidate mixture contains a photoreactive group, said method comprising:

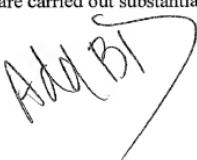
- a) contacting said candidate mixture with said protein, wherein nucleic acids having an increased affinity to the protein relative to the candidate mixture form nucleic acid-protein complexes with the protein;
- b) irradiating said complexes, wherein said nucleic acid-protein photocrosslink;
- 5 c) partitioning the photocrosslinked nucleic acid-protein complexes from the remainder of said candidate mixture; and
- d) identifying a nucleic acid ligand that photocrosslinked to the protein; wherein steps (a)-(c) are performed at one or more work stations on a work surface by a cartesian robotic manipulator controlled by a computer.

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19. A method for identifying a photocrosslinking nucleic acid ligand of a protein from a candidate mixture of nucleic acids, said method comprising:

- a) contacting said candidate mixture with said protein, wherein nucleic acids having increased affinity to the protein relative to the candidate mixture form nucleic acid-protein complexes with the protein;
- b) partitioning the complexed increased affinity nucleic acids from the remainder of the candidate mixture;
- c) amplifying the increased affinity nucleic acids to yield a ligand-enriched mixture of nucleic acids;
- 15 d) incorporating photoreactive groups into said amplified increased affinity nucleic acids;
- e) repeating step a;
- f) irradiating said increased affinity nucleic acids, wherein said nucleic acid-protein complexes photocrosslink;
- 20 g) repeating steps c) and d); and
- h) identifying a photocrosslinking nucleic acid ligand to the protein; wherein steps (a)-(g) are carried out substantially by automated machines controlled by a computer.

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